Title: **MICROTOX BASIC TEST (PHENOL STANDARD)**

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1.0 OBJECTIVE

This method tests a standard toxicant, whose test results are well documented, and also checks the performance of the complete Microtox® system. The advantage of using phenol is that its toxicity is extremely well characterized and produces a clear effect rapidly.

2.0 HEALTH AND SAFETY

Personnel should wear lab coats, lab aprons, safety goggles, and chemical resistant gloves when preparing chemical stocks, and when dosing with test chemicals or effluents.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This method should be restricted to use by or under the supervision of professionals experienced in toxicity testing.

4.0 REQUIRED AND RECOMMENDED MATERIALS

Microbics Model 500 Analyzer	Microtox® Test Reagent
Microtx® Reconstitution Solution	Phenol Standard
2.5% NaCl Diluent	22% NaCl Diluent
Test Cuvettes	Lab coat
Gloves	Repeat Pipettor
0.6ml Syringe	Pipet Tips
Sodium Chloride	1000-ml Pipettor

500-ml Pipettor
100-ml Glass Beaker
1-L Volumetric Flask
250-ml Pipettor
De-ionized Water (DI H_2O)

5.0 PROCEDURE

5.1 Preparation

5.1.1 Analyzer and Incubator Preparation

- Plug in the Microbics M500 Toxicity Analyzer.
- Place a cuvette in the REAGENT well.

5.1.2 Making Phenol Standard

- Weigh out 0.025g of Phenol (located in Rm. 230) into a 100-ml glass beaker.
- Add 100 ml of DI water.
- Pour into a 250-ml volumetric flask and mix well.
- Pour into clean amber bottle.
- Date and label the bottle.

5.1.3 Making 2.5% NaCl Diluent

- Weigh out 25g of microbiology grade Sodium Chloride (located in Rm. 230) into a 50-ml glass beaker.
- Pour the NaCl into a 1-L volumetric flask.
- Add DI water to the mark on the neck of the flask.
- Cap with a stopper and invert to mix.
- Pour into a pre-clean 1000-ml Pyrex Culture Bottle.
- Date and label the bottle

5.1.5 Making 22% NaCl Diluent

- Weigh out 220g of Microbiology Grade Sodium Chloride (located in Rm. 230) into a 100-ml glass beaker.
- Pour the NaCl into a 1-L volumetric flask.
- Add DI water to the mark on the neck of the flask.
- Cap with a stopper and invert to mix.
- Pour into a pre-clean 1000-ml Pyrex Culture Bottle.
- Date and label the bottle

5.2 Phenol Standard Test

- Phenol Standard Test should be conducted at least once a week when running samples.
- Add 2-ml of Reconstitution Solution (water) (Beige Refrigerator in Rm. 230) to a cuvette and place it in REAGENT well.
- Take Microtox Reagent vials out of Freezer A and place them in the Beige Refrigerator's Freezer.
- Take out Phenol Standard, 2% and 22% NaCl diluents from the Beige Refrigerator.
- Place cuvettes in Rows A & B of the Microbics M 500 Analyzer.
- Pipette 500µl of 2% NaCl diluent into cuvettes in Row B for 5 mins.
- Pipette 1ml of 2% NaCl solution into cuvettes 1-4 in Row A.
- Pipette 250µl of 22% NaCl in to A5.
- Pipette 2.5ml of Phenol Standard in to A5. Mix 3-4 times with pipette. Use the 500µl-pipettor first to add 0.5 ml standard, then use the 1ml-pipettor (broken one) to add 2ml of standard.
- Transfer 1ml from A5 to A4. Mix three times. Transfer 1ml from A4 to A3. Mix three times. Transfer 1ml from A3 to A2. Mix three times.
- Discard 1ml from A2 into a beaker appropriately labeled "Spent Waste". Using the 250µl-pipettor, discard 750µl from A5.
- When timer reaches 5 minutes, reconstitute 2 vials of reagent. Remove the foil from the vials and add the 2-ml of Reconstitution Solution from the REAGENT cuvette to them. Cap the vials and invert several times to dissolve the reagent. Pour the solution back into the cuvette and place it back in the REAGENT well. Try to get everything out of the vials by using the repeat pipettor. Attach a 0.6ml syringe to the pipettor and get all of the reagent out of the vials.
- Mix reagent ~20 times with a 1ml-pipettor.
- Using the repeat pipettor with a 0.6ml syringe, add 10µl of reagent to each of the cuvettes in Row B. Mix by shaking it by hand.
- Set timer for 15 minutes.
- After everything is set up for this test, use the remainder of the 15 mins to start setting up the next sample.
- Set up computer for a BASIC TEST:

  1 CONTROL
  4 DILUTIONS
  45 = INITIAL CONC.
  2 = DILUTION FACTOR

MG/L = UNITS
5 MINS. TEST TIME (Delete the 15 min.)
Zero time readings should be (✓)ed

- After 15 mins, place cuvette B1 in READ well and press the SET button.
- When the green light comes back on, touch the space bar and take initial readings, as prompted by the computer.
- Transfer 500µl from A1 to B1, A2 to B2, A3 to B3, A4 to B4 and A5 to B5. Pipet tip does not need to be changed between transfers.
- Touch space bar and wait for the remainder of the 5 mins. to expire.
- After 5 mins. expires, take readings as prompted by the computer.
- After taking the readings, check results: EC_{50} = 13-26 mg/L, Coefficient of Determination $\geq$ 0.95, Confidence Factor should be between 1 and 2.
- If the results are not within these parameters, another phenol test needs to be conducted again.

5.3 End of the Test

The cuvette contents are disposed of in the sink and the cuvettes are thrown away in the BROKEN GLASS DISPOSAL by the door.

6.0 QUALITY ASSURANCE/QUALITY CONTROL

Personnel should follow good laboratory practices during Microtox® testing.

7.0 REFERENCES

Microtox® Manual. Microbics Corporation. 1992. Carlsbad, CA. 476 pp.